CLAIMS

1. A method of sequencing a target polynucleotide comprising the steps of:
(a) Carrying out template derived nucleotide synthesis utilising a labelled nucleotide;
(b) detecting the presence or absence of said labelled nucleotide;
(c) replacing said labelled nucleotide with an unlabelled nucleotide; and
(d) repeating steps a) to c)
with the proviso that if said labelled nucleotide is labelled with a label directly attached to the nucleotide, then the replacement of said labelled nucleotide comprises removal of the whole of said labelled nucleotide and replacement with an unlabelled nucleotide, and only said labelled nucleotide can be removed.

2. A method as claimed in claim 1 wherein said target polynucleotide is attached to a solid surface.

3. A method as claimed in claim 1 or claim 2 wherein said labelled nucleotide is labelled with a fluorescent tag.

4. A method as claimed in claim 3 wherein said fluorescent tag is attached directly to said nucleotide.

5. A method as claimed in claim 3 or claim 4 wherein said lableed nucleotide is attached to a quencher at the gamma position, and said fluorescent tag is attached at the 3' position or to the base.

6. A method as claimed in claim 4 or claim 5 wherein step (c) comprises chemically inactivating or photobleaching said fluorescent tag.

7. A method as claimed in claim 4 wherein step (c) comprises removal of said labelled nucleotide and replacement with an unlabelled nucleotide, wherein said unlabelled nucleotide is a degradation resistant nucleotide.

8. A method as claimed in any one of claims 1 to 4 wherein said labelled nucleotide is a degradation labile nucleotide.

9. A method as claimed in anyone of claims 1 to 3 wherein said labelled nucleotide is labelled with a nanoparticle.

10. A method as claimed in claim 9 wherein said nanoparticle is a semiconductor nanocrystal.

11. A method as claimed in any one of claims 3, 9 or 10 wherein said fluorescent tag or said nanoparticle are attached to said labelled nucleotide by a linkage.

12. A method as claimed in claim 11 wherein said linkage comprises a binding pair.

13. A method as claimed in claim 12 wherein said binding pair comprises streptavidin and biotin or an analog thereof.

14. A method as claimed in claim 13 wherein said biotin or analogue thereof is 2-Iminobiotin or Desthiobiotin.

15. A method as claimed in claim 12 or claim 13 wherein said fluorescent tag or nanoparticle is conjugated to said streptavidin.

16. A method as claimed in any one of claims 11 to 13 wherein said linkage comprises a cleavable bond.

17. A method as claimed in any one of claims 12 to 16 wherein step (b) comprises incorporation of an unlabelled nucleotide adapted for the attachment of a fluorescent tag or nanoparticle; and attaching said fluorescent tag or nanoparticle to said unlabelled nucleotide.

18. A method as claimed in any one of claims 9 to 17 wherein step (c) comprises removing the fluorescent tag or nanoparticle from said labelled nucleotide.

19. A method as claimed in claim 18 wherein said fluroescent tag or nanoparticle is removed from said labelled nucleotide by cleaving the cleavable bond in the linkage attaching said fluorescent tag or nanoparticle to the nucleotide.

20. A method as claimed in claim 15 wherein said linkage attachs one member of a binding pair to the nucleotide, and the other member of the binding pair is attached to said nanoparticle.

21. A method as claimed in claim 9 wherein said linkage comprises a binding member attached by a cleavable bond to said nucleotide and the other binding member is attached to said fluorescent tag.

22. A method as claimed in claim 21 wherein step (d) comprises removal of said fluorescent tag by cleaving said cleavable bond.

23. A method as claimed in anyone of claims 1 to 3 wherein step (b) is carried out by means of an imaging technique utilising FRET (fluorescent resonance energy transfer).

24. A method as claimed in claim 23 wherein said target polynucleotide is treated with a DNA stain.

25. A method as claimed in claim 23 or claim 24 wherein said labelled nucleotide is labelled with a label that acts as a FRET partner.

26. A method as claimed in anyone of claims 23 to 25 wherein steps (a)-(c) occur simultaneously.

27. A method as claimed in any one of claims 1 to 3 wherein said labelled nucleotide is an oligonucleotide and step (a) comprises ligating said oligonucleotide to a primer annealed to said target polynucleotide.

28. A method as claimed in claim 27 wherein step (c) comprises contacting said oligonucleotide with a degradation agent to remove the label.

29. A method as claimed in claim 27 or claim 28 wherein said ligation forms a degradation resistant bond.

30. A method as claimed in any one of claims 27 to 29 wherein said oligonucleotide comprises a degradation labile intranucleoside bond and step (c) comprises contacting said oligonucleotide with an agent that degrades said degradation labile intranucleoside bond.

31. A method as claimed in claim 30 wherein said degradation labile intranucleoside bond is between the terminal nucleotide which is ligated to said primer and the adjacent nucleotide.

32. A method as claimed in any one of claims 27 to 29 wherein the terminal nucleotide which is ligated to the primer is a deoxynucleotide, and at least the adjacent nucleotide is a ribonucleotide.

33. A method as claimed in claim 27 wherein said oligonucleotide comprises the structure:

Terminal nucleotide – N - nucleotide attached to a label – M – nucleotide attached to a quencher

Wherein N and M are each independently a bond or at least one nucleotide; and M comprises a first degradation labile intranucleoside bond.

34. A method as claimed in claim 33 wherein said ligation forms a degradation resistant bond.

35. A method of sequencing a target polynucleotide comprising the steps of:

(a) Carrying out template derived nucleotide synthesis by ligating an labelled oligonucleotide to a primer annealed to said target polynucleotide, wherein said ligation form a degradation resistant bond , and wherein said oligonucleotide comprises the structure:

Terminal nucleotide – N - nucleotide attached to a fluorescent label – M – nucleotide attached to a quencher

wherein N and M are each independently a bond or at least one nucleotide; and M comprises a first degradation labile intranucleoside bond ;

(b) Contacting said oligonucleotide with a first degradation agent;

(c) Detecting the presence or absence of said labelled oligonucleotide;

(d) Contacting said oligonucleotide with a second degradation agent;and

(e) Repeating steps (a)-(d)

36. A method as claimed in claim 33 or claim 34 wherein N comprises a second degradation labile intranucleoside bond, wherein said second degradation labile intranucleoside bond is resistant to the degrdation agent used to degrade the first degradation labile intranucleoside bond.

37. A method of sequencing a target polynucleotide comprising the steps of:

(a) Carrying out template derived nucleotide synthesis by ligating an labelled oligonucleotide to a primer annealed to said target polynucleotide, wherein said ligation form a degradation resistant bond , and wherein said oligonucleotide comprises the structure:

Terminal nucleotide – N - nucleotide attached to a fluorescent label – M – nucleotide attached to a quencher

wherein N and M are each independently a bond or at least one nucleotide; and M comprises a first degradation labile intranucleoside bond; and N comprises a second degradation labile intranucleoside bond, wherein said second degradation labile intranucleoside bond is resistant to the degradation agent used to degrade the first degradation labile intranucleoside bond;

(b) Contacting said oligonucleotide with a first degradation agent;
(c) Detecting the presence or absence of said labelled oligonucleotide;
(d) Contacting said oligonucleotide with a second degradation agent;and
(e) Repeating steps (a)-(d)

38. A method as claimed in claim 27 wherein said oligonucleotide comprises the structure:

Terminal nucleotide- N- nucleotide attached to a fluorescent label – L – nucleotide attached to a quencher

Wherein N is a bond or at least one nucleotide; and L comprises a number of nucleotides which together form a hairpin structure when said oligonucleotide is not annealed to said template.

39. A method as claimed in claim 38 wherein N comprises a degradation labile intranucleoside bond.

40. A method as claimed in claim 38 wherein said ligation forms a degradation resistant bond.

41. A method as claimed in any one of claims 1 to 40 wherein said target polynucleotide forms part of an array.

42. A method as claimed in claim 39 wherein step (b) comprises measuring the signal generated by a plurality of said labelled nucleotides.

43. A method as claimed in claim 39 wherein step (b) comprises detecting the presence or absence of said labelled nucleotide for each individual polynucleotide.

44. A method as claimed in claim 43 wherein said detection is carried out by means of single DNA molecule imaging.

45. A method as claimed in claim 44 wherein said single DNA molecule imaging technique is fluorescence resonance energy transfer (FRET).

46. A method as claimed in claim 45 wherein said polynucleotide is treated with a DNA stain.

47. A method as claimed in claim 46 wherein the label on said labelled nucleotide acts as a FRET partner to said DNA istain.

48. A method of comparing two or more polynucleotide sequences comprising:

a) differentially labelling the nucleotide sequences being compared;
b) immobilising said nucleotide sequences on a surface;
c) detecting the locus of each nucleotide sequence; and
d) sequencing said polynucleotide sequences using a method as claimed in any of claims 1 to 47.

49. A method as claimed in claim 48, further comprising photobleaching the label prior to the sequencing of said polynucleotide sequence.

50. A method of resolving ambiguities in a polynucleotide sequence comprising:
    a) identifying an area of ambiguity in a polynucleotide sequence;
    b) designing probes for each of the suspected sequence possibilities; and
    c) utilising the primers formed to sequence said polynucleotide sequence utilising a method as claimed in any of claims 1 to 47.

51. A method of sequencing mRNA comprising:
    a) contacting an array of probes designed to hybridise to mRNA molecules with a sample of mRNA under conditions whereby the mRNA will hybridise to said probes; and
    b) sequencing said mRNA utilising a method as claimed in any one of claims 1 to 47.

52. A method as claimed in 51 wherein said probe is designed to hybridise to the polyadenylation signal, 5' cap, 3' tail or the poly A tail.

53. A method of sequencing a target polynucleotide comprising the steps of:
    (a) treating said target polynucleotide with an intercalating dye;
    (b) extending a primer annealed to said target polynucleotide utilising a nucleotide labelled with a label which acts as a FRET partner to said DNA intercalating dye;
    (c) detecting the presence or absence of said nucleotide by means of an imaging technique that utilises FRET; and
    (d) repeating steps a-c;
    wherein steps (a) and (b) can occur in any order.

54. A method of sequencing a target polynucleotide comprising the steps of:
    (a) extending a primer annealed to said target polynucleotide utilising a labelled nucleotide wherein the label is directly attached to the nucleotide;
    (b) detecting the presence or absence of said labelled nucleotide within said extended primer;

(c) removal of said labelled nucleotide, and replacement of said labelled nucleotide with an unlabelled degradation resistant nucleotide; and

(d) repeating steps a-c;

wherein the 3' end of said primer comprises at least one degradation resistant nucleotide.

55. A method of sequencing a target polynucleotide comprising the steps:

(a) extending a primer annealed to said target polynucleotide utilising a labelled nucleotide wherein the label is attached to the nucleotide via a cleavable linkage;

(b) detecting the presence or absence of said labelled nucleotide within said extended primer;

(c) cleaving said label from said nucleotide; and

(d) repeating steps a-c.

56. A method of sequencing a target polynucleotide comprising the steps of;

(a) extending a primer annealed to said target polynucleotide using a nucleotide attached by a cleavable linkage to one member of a binding pair;

(b) contacting said nucleotide with a label attached to the other member of a binding pair under conditions such that the two members of the binding pair bind to one another;

(c) detecting the presence or absence or said label;

(d) removal of said label and said binding pair by cleaving said cleavable linkage; and

(e) repeating steps a-d.

57. A method of sequencing a target polynucleotide, comprising the steps of:

(a) carrying out template derived polynucleotide synthesis utilising a nucleotide labelled with a FRET partner and at least one other polymerisation reaction component labelled with a FRET partner;

(b) determining the nucleotide incorporated by detecting FRET interactions; and

(c) repeating steps (a) and (b).